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厦门大学

硕士学位论文

海洋放线菌 *Brevibacterium* sp. BS01 杀藻活性物质的分离鉴定及其对塔玛亚历山大藻作用机理的研究

Isolation and identification of algicidal substances produced by *Brevibacterium* sp. BS01 and algicidal mechanism of algicidal compound against *Alexandrium tamarense*

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摘 要

赤潮的控制与防治对于保证海洋环境安全和海洋生态健康是至关重要的。本论文主要研究了海洋杀藻放线菌 BS01 的种属关系；根据 BS01 杀藻活性物质的基本理化性质，分离纯化得到一个具有杀藻活性的化合物；同时探讨了塔玛亚历山大藻在 BS01 胞外分泌物胁迫下生理生化水平的响应；考察藻细胞死亡过程中细胞形态变化；氧化压力下藻细胞光合系统损伤情况；综合运用 real-time PCR 技术研究了藻细胞在杀藻活性物质作用下靶功能基因 *psbA*、*psbD*、*hsp*、*cox* 和 *cob* 转录水平变化。主要研究结果如下：

（1）分离鉴定得到一株具高效稳定杀藻功能的放线菌，菌株为杆状，属于放线菌目，短杆菌属 *Brevibacterium* sp. （Genbank No. GQ274005），并将其命名为 BS01。

（2）从 BS01 的发酵培养物中分离鉴定到一种杀藻化合物（2-异丁氧基苯基）胺（(2-isobutoxyphenyl)amine），分子量大小为 165。藻细胞在 5 μg/mL~20 μg/mL 浓度下比较敏感，藻细胞就静沉于瓶底部，光学显微镜下观察失去游动能力，但细胞形态维持完整性，无细胞裂解。

（3）BS01 上清液使藻细胞运动性丧失，质壁分离，藻细胞裂解，叶绿体等细胞器逐渐溶解，内容物溶出，最终整个细胞完全溶解，呈空泡化，推测其经历细胞坏死的过程。在激光共聚焦显微镜下，观察到少数细胞经历细胞程序性死亡的过程，细胞质紧致，细胞核形成类核小体，并呈现趋边化等现象。

（4）细胞内部活性氧（ROS）短时间内显著增加，其体内抗氧化酶体系活性随处理时间和浓度的增加而升高，膜脂质氧化加重，DNA 电泳图像表现出断裂弥散现象。叶绿素 a 和类胡萝卜素降解，12 h 时高浓度处理组（1.5%）的光能转换效率明显低于对照，约为对照的 60%。短时间内（2 h），藻细胞 ETR 与对照相比皆下降，随着 PAR 的增加，所有处理组的电子传递速率都达到饱和，随处理时间的延长，出现光抑制现象。在 8 h 时，0.5%处理组的热能散失显著增加，是其他组的将近 2.2 倍。

（5）荧光定量 PCR 结果表明，*cob* 和 *cox* 在低浓度杀藻活性物质处理下，基因转录水平受到抑制，随着处理浓度的增加，其转录水平也在逐渐的增加；*psbA*

和 *psbD* 分别编码光合系统中两个重要的 D1 和 D2 蛋白，其荧光定量 PCR 结果表明，藻细胞光合作用系统受到损伤，处理初期其转录受到抑制，后期逐渐增加，以抵抗外界不良环境所造成的胁迫。*hsp* 是一种应激蛋白，在本研究中，其在短时间内没有表现出即时高表达的响应，而是在系列浓度杀藻活性物质处理下，其转录水平皆低于对照。

关键词：杀藻放线菌；杀藻化合物；基因转录；塔玛亚历山大藻

Abstract

Control of red-tide is becoming vital for marine security and ecological recovery. Marine actinomycetes are playing an irreplaceable role on marine biodiversity and stability of marine ecosystem, providing a possible path for controlling red-tide.

In this paper, the typical harmful alga, *Alexandrium tamarense* (ATGD98-006), and algicidal actinomycete *Brevibacterium* sp. BS01 were studied as dominant materials. This study focused on isolation and identification of actinomycetes with the capacity of controling algae and explorition of algicidal mechanism. Based on physical and chemical characters, a novel algicidal compound was isolated from the culture of BS01. Physiological and biochemical response of algal cells under the stress of supernatant of BS01 was investigated and morphologic changes were observed in the process of cell death. Besides, plant photosynthetic function was explored with the chlorophyll fluorescence kinetics technique. Also, the study included the investigation of expression of target functional genes (*psbA*、*psbD*、*hsp*、*cox*and *cob*) by real-time PCR. There are following main results achieved from these experiments:

(1) A bacterial strain BS01 was isolated and identified from the blooms in Xiamen sea areas and classified as *Brevibacterium* sp. of actinomycete based on microbiological characters and molecular analysis. It was rod-shaped and its Genebank No. was GQ274005.

(2) The co-utilization of silica gel column and gel chromatography mass spectrum as well as nuclearmagnetic resonance imaging technique, made (2-isobutoxyphenyl)amine purified from culture of BS01. It was a hydrophobic liquid compound with aniline and a small molecular weight of only 165. *Alexandrium tamarense* was sensitive to 5 μg/mL of (2-isobutoxyphenyl)amine with the loss of mitibility and sinking at the bottom of flasks, while cells kept intact even under the concentration of 20 μg/mL. With the extension of treatment time as well as increasing concentration of supernatant, algicidal effect displayed continuingly obvious.

（3）The death process of algae was monitored with the help of various microscopes, and algal cells were partly clarified as death in the form of necrosis: loss of swimming, occurance of plasmolysis, lysis of cell wall and membrane, organelles gradually dissolving with efflux of cell contents and whole cell completely dissolving as well as severe vacuolization. Programmed cell death (PCD) was also observed under the laser scanning confocal microscope (LSCM), in which individual algal cells literally destroyed themselves with the production of nucleoid and gradual marginalization.

（4）Reactive oxygen species (ROS) were greatly produced in a short time as a result of attack of active chemical compounds from BS01. Antioxidant enzymes activities were motivated with the adding of concentration of supernatant and extension of treatment time. And ROS could react directly with cellular lipids and DNA, and cause cell oxidative damage, dysfunction, smear of DNA. Oxidative damage to algal cells was thought to be one of main causes of cell death. Meanwhile, the amount of pigments continuingly discreased and photosynthesis was severely suppressed, resulting in impeding electron transport and ATP formation.

（5）Real-time PCR of *cob* and *cox* showed the similar trend that transcription was impeded when algal cells were treated at low concentration of algicidal compounds, while at high concentration of supernatant from BS01, it showed a continuingly rising expression level. Gnens *of psbA* and *psbD* code proteins D1 and D2 of photosynthetical system, respectively, which are playing vital role in photosynthesis. The results of real-time PCR demonstrated photosynthetic system in *Alexandrium tamatense* was destroyed at the intial stage, and then transcription of the two genes arose with the rising of supernatant concentration, aiming at resisting the adverse environmental impacts. In addition, *hsp* is the gene of coding heat stress protein, which didn’t show instantly high expression, but experienced a lower level than control.

Key words: algicidal actinomycetes; algicidal compound; gene transcription; *Alexandrium tamatens*